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Microbial bioreductions of γ- and δ-ketoacids and their esters

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Abstract—A series of yeasts were used in the bioreductions of aliphatic and aromatic γ- and δ-ketoacids and esters to investigate the preparation of enantiomerically pure γ- and δ-lactones through the intermediacy of their corresponding γ- and δ-hydroxyacids and esters. Bioreduction of ethyl 4-oxononanoate **2a** with *Pichia etchellsii* afforded the γ-nonanolide (+)-**5a** with 99% e.e., while *Pichia minuta* proved to be the best choice for the bioreduction of ethyl 2-oxocyclohexylacetate **2e**, which afforded *cis*-(−)-**5e** and *trans*-(−)-**5e** with 98 and 99% e.e., respectively. Reduction of 3-(2-oxocyclohexyl)propionic acid **3e** with *Pichia glucozyma* gave predominantly the corresponding δ-lactone *trans*-(−)-**6e** with 94% e.e., whose absolute configuration was determined by means of CD spectroscopy.

1. Introduction

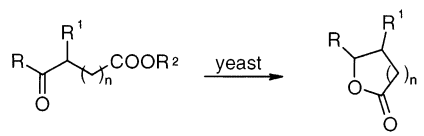
Substituted γ- and δ-lactone rings are present in many of the optically active natural products isolated from insects, plants, fungi and marine organisms.[1,2] Therefore, the synthesis of these heterocyclic subunits in enantiomerically pure form has received particular attention. Of the numerous synthetic possibilities,[3] the enantioselective reduction of their corresponding γ- or δ-ketoacid or ester intermediates by means of enzymes, either isolated or belonging to a whole cell system, has found general applicability.[4] It is widely accepted that baker's yeast plays the most traditional and useful role,[4] although yeasts belonging to other genera[5] have been found to be able to compete with the more common *Saccharomyces cerevisiae*. In this paper, we report the results obtained for the bioreduction of some γ- and δ-ketoacids and their corresponding ethyl esters with *S. cerevisiae* and other yeasts such as *Pichia minuta* CBS 1708, *Pichia fermentans* DPVPG 2770, *Pichia glucozyma* CBS 5766, *Pichia etchellsii* CBS 2011, *Candida boidinii* CBS 2428, *Candida utilis* CBS 621 and *Kluyveromyces marxianus* CBS 397, in order to make a comparison of their respective efficiency in the biotransformations.

2. Results and discussion

The substrates chosen in this study are the linear aliphatic and aromatic γ-ketoacids **1a** and **1c**, their respective ethyl esters **2a** and **2c**, and their homologs **1b** and **1d** and **2b** and **2d**, all bearing a methyl group at the β carbon atom. The cyclic γ-ketoacid **1e**, its ethyl ester **2e** and the cyclic δ-ketoacid **3e** and its ester **4e** were also studied (Scheme 1). The aim was to examine the influence of the aromatic ring linked to the site of the reaction and also the influence of the smallest of the possible substituents at the carbon atom adjacent to the carbonyl group on the enzymatic activity.

2.1. Bioreductions of 1a, 1b, 2a and 2b

Table 1 lists the main results obtained for bioreductions of **1a**, **1b**, **2a** and **2b**. In the case of reductions with *S.*



R^2 = H, n = 1: **1a-e**
R^2 = Et, n = 1: **2a-e**
R^2 = H, n = 2: **3e**
R^2 = Et, n = 2: **4e**

n = 1: **5a-e**
n = 2: **6e**

	R	R^1
a	C_5H_{11}	H
b	C_5H_{11}	Me
c	Ph	H
d	Ph	Me
e	$(CH_2)_4$	

Scheme 1.

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cerevisiae several conditions were examined,[4a] such as the use of dry baker's yeast, pre-treated raw baker's yeast and reactions with addition of glucose, while the remaining yeasts were grown as reported before[6] and used as re-suspended cells.

As can be seen in Table 1, the acids **1a** and **1b** were reduced much less easily than the corresponding ethyl esters **2a** and **2b**, but reductions of **2a** and **2b** were complicated by the competing hydrolysis of the ester group. In some cases, for instance in the transformation of **2a** with *C. boidinii*, the ketoacid was obtained as nearly the sole product. Hydrolyses of **2b** were only slightly enantioselective and the resulting acid (+)-**1b** could be recovered with 20–39% e.e. In contrast, when the enzymes were effective in reducing the substrates, the enantioselectivity was in general high, ranging from 62 to 99%, whereas diastereoselectivity was poor. In fact from the reduction of **2b** both *cis*- and *trans*-diastereomers of **5b** were obtained.

In the reductions of **1a** and **2a**, good results were obtained with baker's yeast[7,8] and with *P. etchellsii*, which showed the same enantiopreference, affording (*R*)-(+)-**5a**[9] with very high e.e.s (Table 1). Interestingly, the reduction of **2a** performed with *P. glucozyma* afforded the enantiomeric (*S*)-(−)-**5a**, although with low enantiomeric purity.

Reductions of **1b** and **2b** with baker's yeast have already been reported:[7,8,10] the *trans*-lactone (4*S*,5*R*)-(+)-**5b** was obtained from **1b** as a single isomer and the *cis*-lactone (4*S*,5*S*)-(−)-**5b** from **2b**, although as a 7:3 mixture with *trans*-(−)-**5b**. Since these diastereomeric γ-lactones constitute the *Cognac* lactones[11] their preparation in enantiomerically pure form is of interest. *P. glucozyma* was the only other microorganism to give fairly good results in the bioreduction of **2b**, affording *trans*-(−)-**5b** with 96% e.e., as a mixture with its enantiomeric *cis*-(−)-**5b** having 62% e.e.

2.2. Bioreductions of 1c, 1d, 2c and 2d

Bioreductions of compounds **1c** and **2c** with baker's yeast have already been reported to furnish the lactone (*S*)-(−)-**5c** with >95% e.e.[12] Bioreductions of the same substrates with the other yeasts were unsatisfactory, as conversions were low and in the case of the ester **2c** hydrolysis was always a competing reaction. The only good result was that obtained with *K. marxianus* on **1c** (Table 2), which gave (*S*)-(−)-**5c** with 70% yield and 99% e.e. All active yeasts showed the same enantiopreference for the (*S*)-configuration of the carbinol carbon atom.

Similarly, reductions of the more hindered systems **1d** and **2d** were unsatisfactory and most yeasts did not work at all. Only baker's yeast was able to mediate the reduction of **1d** in a diastereoselective manner (71% d.e.) affording *trans*-**5d** with 99% e.e., although in low yield. The synthesis of the lactone *trans*-(−)-**5d** is notable since it has recently been proposed as an intermediate in the synthesis of a prototype non-peptidic inhibitor of the enzyme rennin.[13] Ester **2d** was reduced successfully only by *P. glucozyma*, which afforded a 1:3 mixture of *cis*- and *trans*-lactones **5d** both having low e.e.s.

2.3. Bioreductions of 1e–4e

With the cyclic γ- and δ-ketoacids and esters **1e**–**4e** almost all bioreductions proceeded to fairly high conversion values, with an enantioselectivity ranging from 26 to 99% and a diastereoselectivity ranging from 5 to 97% (Table 3). Baker's yeast reductions of **2e**,[14] **3e**[15] and **4e**[15] has already been reported to give very good results in terms of conversion and enantioselectivity and with **4e** also in terms of diastereoselectivity. On the contrary, the reduction of the ketoacid **1e** with *S. cerevisiae* was not so satisfactory affording both diastereomers of **5e** with moderate e.e.s. As to the other yeasts, *P. minuta* and *P. fermentans* were active towards **2e** affording the *cis*- and *trans*-diastereomers of **5e**, (3a*S*,7a*S*)-(−)-**5e** and (3a*S*,7a*R*)-(−)-**5e**, respectively, both with high e.e.s, although a small amount of the hydrolysis product was also detected in both cases.

Bioreductions of **3e** and **4e** allowed the isolation of both *cis*- and *trans*-isomers of the δ-lactone **6e** in enantiomerically pure form. *P. minuta* reduced **4e** rapidly and completely to the corresponding isomeric lactones (4a*S*,8a*S*)-(−)-**6e** and (4a*S*,8a*R*)-(−)-**6e** in about 1:1 ratio, both with 99% e.e. *P. glucozyma* was also effective in reducing **3e** and **4e** affording the *trans*-isomer (−)-**6e** with 99% e.e. although as a mixture with the *cis*-isomer, whose enantiomeric purity was lower. Reduction of **3e** with *P. glucozyma* was then used on a preparative scale to isolate *trans*-(−)-**6e** for CD spectroscopy analysis.

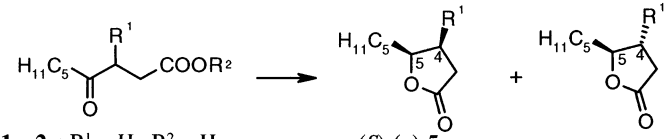
2.4. Determination of the absolute configuration

The (*R*)-configuration of the 4-nonanolide (+)-**5a** was determined by comparison with an authentic commercial sample. The absolute configurations of *cis*-(−)-**5b**,[10] *trans*-(+)-**5b**,[10] (−)-**5c**,[12] *cis*-(−)-**5e**,[14,16] *trans*-(−)-**5e**[14,16] and *cis*-(−)-**6e**[17] are already known, whereas those of the diastereomeric lactones **5d** could not be determined because they were inseparable on column chromatography. The δ-lactone *trans*-(−)-**6e** was assigned (4a*R*,8a*S*)-absolute configuration by comparing its CD spectrum with those of the known lactones (4a*S*,6*S*,8a*S*)-(+)-**7e**[15] and (4a*S*,6*S*,8a*S*)-(−)-**8e**[17] (Fig. 1). The different descriptor for C-(4a) in the three lactones is due to a different priority sequence.

3. Conclusions

Among the yeasts examined, *S. cerevisiae* was the most versatile because it was active with the aliphatic and aromatic ketoacids and esters with only a few exceptions. The enantioselectivity was consistently high and the diastereoselectivity was also good. In some cases,

Table 1. Bioreductions of **1a**, **1b**, **2a** and **2b**[a]



1a, 2a: R^1 = H, R^2 = H, **1b, 2b**: R^1 = Me, R^2 = H, Et

(*S*)-(−)-**5a**

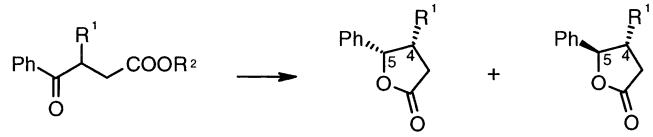
(4*S*,5*S*)-(−)-**5b**

(4*R*,5*S*)-(−)-**5b**

Yeasts	**1a**		**2a**			**1b**			**2b**			
	Conv. (%)[b]	Products	Conv. (%)[b]	Products		Conv. (%)[b]	Products		Conv. (%)[b]	Products		
		Sign of α rel. (%) [e.e. (%)][c]		Sign of α rel. (%) [e.e. (%)][c]			Sign of α rel. (%) [e.e. (%)][c]			Sign of α rel. (%) [e.e. (%)][c]		
		5a		**1a**	**5a**		*cis*-**5b**	*trans*-**5b**		**1b**	*cis*-**5b**	*trans*-**5b**
S. cerevisiae[d]	40	(+) 40 [99]	50	4	(+) 46 [99]	36	–	(+) 36 [99]	71	–	(−) 50 [99]	(−) 21 [83]
P. minuta[e]	0	–	25	22	– 3 [n.d.]	0	–	–	79	(+) 77 [20]	–	– 2 [n.d.]
P. fermentans[e]	5	(+) 5 [99]	100	66	(+) 34 [99]	0	–	–	2	–	–	2 [n.d.]
P. glucozyma[e]	0	–	93	–	(−) 93 [36]	0	–	–	69	–	(−) 17 [62]	(−) 52 [96]
P. etchellsii[e]	37	(+) 37 [90]	90	–	(+) 90 [99]	4	2 [n.d.]	2 [n.d.]	97	(+) 81 [39]	– 5 [n.d.]	(+) 11 [90]
C. boidinii[e]	4	– 4 [n.d.]	95	91	– 4 [n.d.]	0	–	–	0	–	–	–
C. utilis[e]	0	–	87	29	(+) 58 [99]	0	–	–	2	–	– 1 [n.d.]	– 1 [n.d.]
K. marxianus[e]	10	(+) 10 [99]	91	53	(+) 38 [65]	4	–	– 4 [n.d.]	19	–	– 1 [n.d.]	(−) 18 [82]

[a] 7 days.
[b] Determined by HRGC.
[c] Determined by chiral HRGC on a γ-CDX column for compounds **5a** and *cis*-**5b** and on a β-CDX column for *trans*-**5b**;
[d] Compound **1a** (0.5 mmol), raw baker's yeast (5 g), glucose (3.1 g), water (10 mL); **2a** (0.5 mmol), dry baker's yeast (2.8 g), glucose (3.1 g), water (19 mL); **1b** (1.8 mmol), baker's yeast (56 g), glucose (56 g), phosphate buffer (0.1 M, pH 7.4, 500 mL), 3 days; **2b** (0.5 mmol), raw baker's yeast (5 g), water (10 mL), 10 days.
[e] Yeast (21–27 mg/mL), substrate (12.5 mg), glucose (0.25 g), phosphate buffer (0.1 M, pH 6.0, 5.0 mL).

Table 2. Bioreduction of **1c**, **1d**, **2c** and **2d**[a]



1c, 2c: R^1 = H R^2 = H, Et
1d, 2d: R^1 = Me R^2 = H, Et

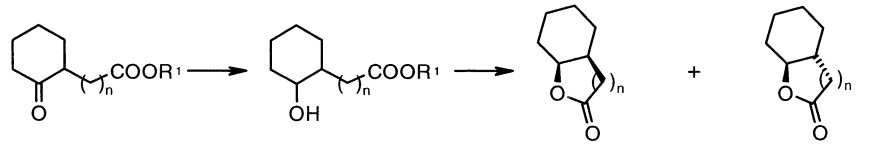
(*S*)-(−)-**5c**
c-**5d**

t-**5d**

Yeasts	**1c**		**2c**			**1d**			**2d**			
	Conv. (%)[b]	Products	Conv. (%)[b]	Products		Conv. (%)[b]	Products		Conv. (%)[b]	Products		
		Sign of α rel. (%) [e.e. (%)][c]		Sign of α rel. (%) [e.e. (%)][c]			Sign of α rel. (%) [e.e. (%)][c]			Sign of α rel. (%) [e.e. (%)][c]		
		5c		**1c**	**5c**		*cis*-**5d**	*trans*-**5d**		**1d**	*cis*-**5d**	*trans*-**5d**
S. cerevisiae[d]	84	(−) 84 [>95]	31	–	(−) 31 [>95]	21	– 3 [n.d.]	– 18 [99]	10	10	–	–
P. minuta[e]	2	– 2 [n.d.]	63	63	–	0	–	–	55	48	– 2 [n.d.]	– 5 [n.d.]
P. fermentans[e]	13	(−) 13 [99]	10	10	–	0	–	–	8	8	–	–
P. glucozyma[e]	21	(−) 21 [99]	40	35	(−) 5 [66]	3	– 3 [n.d.]	–	68	–	– 18 [53]	– 50 [22]
P. etchellsii[e]	18	(−) 18 [96]	45	38	(−) 7 [74]	2	–	– 2 [n.d.]	2	–	–	– 2 [n.d.]
C. boidinii[e]	0	–	26	16	(−) 10 [92]	0	–	–	0	–	–	–
C. utilis[e]	8	(−) 8 [95]	23	10	(−) 13 [91]	0	–	–	9	9	–	–
K. marxianus[e]	70	(−) 70 [99]	37	10	(−) 27 [30]	2	–	– 2 [n.d.]	2	2	–	–

[a] 7 days.
[b] Determined by HRGC.
[c] Determined by chiral HRGC on a β-CDX column.
[d] Ref. 12 for **1c** and **2c**; **1d** or **2d** (0.08 mmol), raw baker's yeast (2.24 g), glucose (2.24 g), phosphate buffer (0.1 M, pH 7.4, 20 mL), 12 days.
[e] Yeast (21–27 mg/mL), substrate (12.5 mg), glucose (0.25 g), phosphate buffer (0.1 M pH 6.0, 5 mL).

Table 3. Bioreduction of **1e**, **2e**, **3e** and **4e**[a]



1e, 2e: n = 1, R^1 = H, Et
3e, 4e: n = 2, R^1 = H, Et

7e

(3a*S*,7a*S*)-(–)-**5e**
(4a*S*,8a*S*)-(–)-**6e**

(3a*S*,7a*R*)-(–)-**5e**
(4a*S*,8a*R*)-(–)-**6e**

Yeasts	**1e**			**2e**				**3e**			**4e**		
	Conv. (%)[b]	Products		Conv. (%)[b]	Products			Conv. (%)[b]	Products		Conv. (%)[b]	Products	
		Sign of α rel. (%) [e.e. (%)][c]			Sign of α rel. (%) [e.e. (%)][c]				Sign of α rel. (%) [e.e. (%)][c]			Sign of α rel. (%) [e.e. (%)][c]	
		cis-**5e**	*trans*-**5e**		**1e**	*cis*-**5e**	*trans*-**5e**		*cis*-**6e**	*trans*-**6e**		*cis*-**6e**	*trans*-**6e**
S. cerevisiae[d]	73	(–) 28 [46]	(+) 45 [70]	72	–	(–) 18 [97]	(–) 54 [97]	75	(–) 30 [99]	(–) 45 [43]	80	(–) 80 [99]	–
P. minuta[e]	26	(–) 12 [93]	(–) 14 [73]	100	23	(–) 44 [98]	(–) 33 [99]	56	(–) 24 [98]	(–) 32 [98]	100[f]	(–) 47 [99]	(–) 53 [99]
P. fermentans[e]	24	(–) 6 [70]	(+) 18 [98]	93	16	(–) 50 [97]	(–) 27 [94]	23	– 5 [n.d.]	(+) 18 [27]	100	(–) 64 [86]	(±) 36 [0]
P. glucozyma[e]	40	(+) 26 [66]	(–) 14 [56]	100	–	(–) 64 [26]	(–) 36 [98]	89	(–) 28 [58]	(–) 61 [99]	100[f]	(–) 55 [80]	(–) 45 [99]
P. etchellsii[e]	80	– 2 [n.d.]	(–) 78 [28]	72	2	(–) 21 [26]	(–) 49 [98]	100[g]	(–) 20 [98]	(–) 80 [87]	100	(–) 47 [93]	(–) 53 [77]
C. boidinii[e]	23	– 2 [n.d.]	(–) 21 [64]	83	–	(–) 25 [59]	(–) 58 [81]	81	– 6 [n.d.]	(–) 85 [92]	83	– 5 [n.d.]	(±) 78 [0]
C. utilis[e]	31	(–) 14 [94]	– 17 [n.d.]	40	3	(–) 20 [61]	(–) 17 [80]	70	– 3 [n.d.]	(–) 67 [72]	12	(–) 9 [60]	– 3 [n.d.]
K. marxianus[e]	74	– 1 [n.d.]	(–) 73 [86]	98	–	(–) 39 [96]	(–) 59 [55]	100	(±) 11 [0]	(–) 89 [30]	93[g]	– 10 [n.d.]	(–) 83 [40]

[a] 7 days.
[b] Determined by HRCG.
[c] Determined by chiral HRGC on a β-CDX column for *cis*-**5e** and a γ-CDX column for *trans*-**5e** and **6e**.
[d] Raw baker's yeast (10 g), water (20 mL), mixture pre-incubated at 50°C for 30 min, **1e** (1 mmol); Ref. 14 for **2e**; Ref. 15 for **3e** and **4e**.
[e] Yeast (21–27 mg/mL), substrate (12.5 mg), glucose (0.25 g), phosphate buffer (0.1 M, pH 6.0, 5 mL).
[f] 1 day.
[g] 3 days.

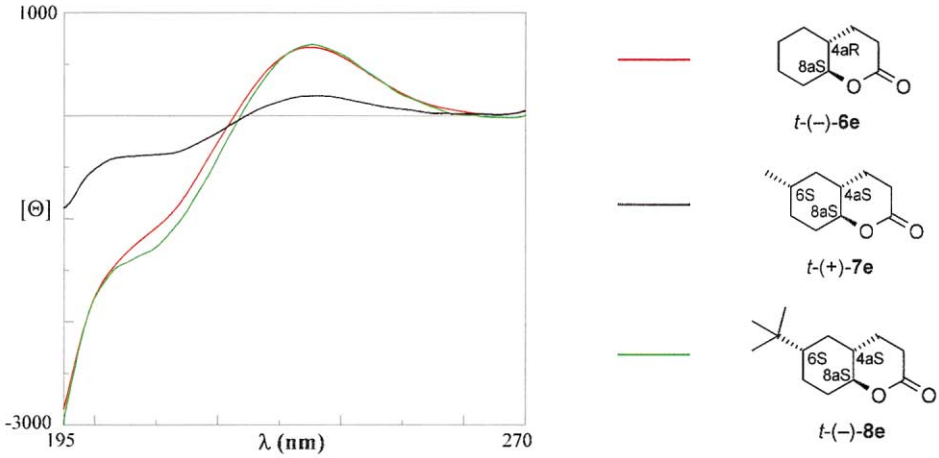


Figure 1. CD spectra of *trans*-(−)-**6e**, *trans*-(+)-**7e** and *trans*-(−)-**8e**.

other yeasts were found to be good alternatives; *K. marxianus*, *P. etchellsii*, *P. glucozyma* and *P. minuta* were effective in reducing **1c**, **2a**, **2b** and **2e**, respectively, with high enantioselectivity but low diastereoselectivity. These microorganisms were often substrate-specific and, when active, did not show a definite trend.

4. Experimental

4.1. General

IR spectra were recorded on a Jasco FT/IR 200 spectrophotometer. ^{1}H and ^{13}C NMR spectra were run on a Jeol EX-400 spectrometer (400 MHz for proton), using deuteriochloroform as a solvent and tetramethylsilane as the internal standard. Coupling constants and W_Hs are given in Hz. Optical rotations were determined on a Perkin–Elmer model 241 polarimeter. CD spectra were obtained on a Jasco J-700A spectropolarimeter (0.1 cm cell). GLC analyses were run on a Carlo Erba GC 8000 instrument and on a Shimadzu GC-14B instrument, the capillary columns being OV 1701 (25 m×0.32 mm) (carrier gas He, 40 KPa, split 1:50) and a Chiraldex™ type G-TA, trifluoroacetyl γ-cyclodextrin (γ-CDX) (40 m×0.25 mm) (carrier gas He, 180 KPa, split 1:100) or DiMePe β-cyclodextrin (β-CDX) (25 m×0.25 mm) (carrier gas He, 110 KPa, split 1:50). TLC was performed on Polygram® Sil G/UV$_{254}$ silica gel pre-coated plastic sheets (eluant: light petroleum–ethyl acetate). Flash chromatography was run on silica gel 230–400 mesh ASTM (Kieselgel 60, Merck). Light petroleum refers to the fraction with a bp of 40–70°C and ether refers to diethyl ether.

4.2. Synthesis of the substrates

Ethyl 4-oxononanoate **2a**[18] was obtained as a by-product in the synthesis of hexanoylsuccinate;[19,20] ethyl 3-methyl-4-oxononanoate **2b** was prepared by radical condensation of ethyl crotonate with hexanal;[10] ethyl 4-oxo-4-phenylbutanoate **2c**[21] was prepared from commercially available 4-oxo-4-phenylbutanoic acid **1c**; ethyl 3-methyl-4-oxo-4-phenylbutanoate **2d** derived from an alkylation reaction of the pyrrolidino enamine of propiophenone with ethyl bromoacetate;[22] ethyl 2-oxocyclohexylacetate **2e** was purchased from Aldrich; ethyl 3-(2-oxocyclohexyl)propionate **4e**[23,24] was obtained from a Micheal-type reaction between ethyl acrylate and the pyrrolidino enamine of cyclohexanone.[17] The acids **1a**,[8,25] **1b**,[10] **1d**,[26] **1e**[27] and **3e**[15,28] were obtained by basic hydrolysis of the corresponding ethyl esters.

4.2.1. Ethyl 4-oxononanoate 2a[18]. Oil; IR (film): 1735, 1710 cm^{-1}; ^{1}H NMR: δ 4.12 (2H, q, J=7.3, CH_2O), 2.72 (2H, t, J=6.5, H-2), 2.57 (2H, t, J=6.5, H-3), 2.45 (2H, t, J=7.6, H-5), 1.61 (2H, quintet, J=7.3, H-6), 1.32 (4H, m), 1.25 (3H, t, J=7.1, CH_3CH$_2$O), 0.89 (3H, t, J=7.1, CH$_3$) ppm; ^{13}C NMR: δ 209.2 (s, C-4), 172.8 (s, C-1), 60.5 (t, CH$_2$O), 42.7 (t, C-5), 36.9 (t, C-2), 31.3 (t), 27.9 (t, C-3), 23.4 (t, C-6), 22.4 (t), 14.1 (q, CH$_3$CH$_2$O), 13.9 (q, C-9) ppm.

4.2.2. Ethyl 3-methyl-4-oxo-4-phenylbutanoate 2d. Compound **2d** was prepared according to the literature.[22] Oil; IR (film): 1715 (COOEt), 1670 (COPh) cm^{-1}; ^{1}H NMR: δ 7.98 (2H, m), 7.56 (1H, m), 7.45 (2H, m), 4.10 (2H, q, J=7.3, CH$_2$O), 3.95 (1H, m, CHCH$_3$), 2.96 (1H, dd, J_1=8.5, J_2=16.8, CH_2COO), 2.46 (1H, dd, J_1=5.9, J_2=16.8, CH$_2$COO), 1.22 (3H, d, J=6.8, CHCH_3), 1.20 (3H, t, J=7.3, CH$_2$CH_3) ppm; ^{13}C NMR: δ 202.7 (s), 172.2 (s), 135.8 (s), 132.9 (d), 128.5 (d), 60.5 (t), 37.4 (t), 37.1 (d), 17.7 (q), 14.0 (q) ppm.

4.3. General procedure for hydrolysis reactions

The ketoester (1 mmol) was added to a solution of KOH (2 mmol) in methanol (1.6 mL). The mixture was stirred for 2 days, acidified with 2N HCl and extracted with ether, after evaporation of the methanol.

4.3.1. 2-Oxocyclohexylacetic acid 1e[27]. Oil; IR (film): 3000 (OH), 1749, 1710 cm^{-1}; ^{1}H NMR: δ 8.40 (1H, OH), 2.82 (2H, m), 2.39 (2H, m), 2.16 (3H, m), 1.89 (1H, m), 1.69 (2H, m), 1.43 (1H, m) ppm; ^{13}C NMR: δ

211.3 (s), 177.9 (s), 46.8 (d), 41.6 (t), 34.2 (t), 33.7 (t), 27.6 (t), 25.0 (t) ppm.

4.4. Synthesis of lactones

Lactones **5a** and **5c** were purchased from Aldrich. Lactones **5b**,[10] **5e**[16,29] and **6e**[17] were prepared according to the literature methods.

4.4.1. 4,5-Dihydro-4-methyl-5-phenyl-2(3*H*)-furanone 5d. To a solution of **2d** (0.220 g, 1 mmol) in EtOH (0.5 mL) was added $NaBH_4$ (0.019 g, 0.5 mmol) with stirring. At the end of the reaction, the solvent was evaporated and the crude reaction mixture was extracted with ether. The organic phase was dried on Na_2SO_4. All spectroscopic data are in accordance with the literature.[26,30]

4.5. Bioreduction conditions

4.5.1. General procedure for baker's yeast reduction. To a stirred suspension of raw baker's yeast or dry baker's yeast in water or 0.1 M phosphate buffer (pH 7.4), was added glucose; the suspension was stirred for 30 min and the γ- or δ-ketoesters or acids were added at rt. The course of the reaction was monitored by HRGC after treatment of the crude reaction mixture with CH_2N_2 to esterify the acid. The e.e. of the lactones was determined by chiral HRGC on a γ-CDX or a β-CDX column. Amounts of baker's yeast and glucose, substrate concentrations and reaction times are indicated in Tables 1–3.

4.5.2. General procedure for yeasts reduction. The following yeasts were used: *P. minuta* CBS 1708, *P. fermentans* DPVPG 2770, *P. glucozyma* CBS 5766, *P. etchellsii* CBS 2011, *C. boidinii* CBS 2428, *C. utilis* CBS 621 and *K. marxianus* CBS 397. To a stirred suspension of wet yeast (5 mL, 21–27 mg/mL of dry weight) in phosphate buffer (0.1 M, pH 6.0), was added glucose (0.25 g). The suspension was stirred for 30 min and the γ- or δ-ketoesters or acids (12.5 mg) added at rt. The course of the reaction was monitored by HRGC after treatment of the crude reaction mixture with CH_2N_2 to esterify the acid. The e.e. of the lactone product was determined by chiral HRGC on a γ-CDX or a β-CDX column.

4.5.3. (4a*R*,8a*S*)-Octahydro-2*H*-1-benzopyran-2-one *t*-6e. To *P. glucozyma* (45 mL, 23 mg/mL) in phosphate buffer (pH 6.0) and glucose, (4.5 g, 0.225 g) was added **3e** (1.3 mmol) and the mixture was stirred at rt. After 4 days the levels of **3e**, *c*-**6e** and *t*-**6e** were 14, 6 and 80%, respectively. Separation by flash chromatography furnished pure *t*-**6e** (0.015 g, 0.1 mmol). IR, ^1H and ^{13}C NMR were identical with the reported values.[31] $[\alpha]_D^{25} = -40.5$ (*c* 0.98, MeOH), $\Delta\varepsilon_{235} = +0.20$, $\Delta\varepsilon_{209} = -0.34$ (MeOH), e.e. = 94% (by chiral HRGC on a γ-CDX column).

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References

1. (a) Rodriguez, A. D.; Piña, I. C.; Barness, C. L. *J. Org. Chem.* **1995**, *60*, 8096–8100; (b) Brown, H. C.; Kulkarni, S. K.; Racherla, U. S. *J. Org. Chem.* **1994**, *59*, 365–369 and references cited therein; (c) *Handbook of Natural Pesticides*; Morgan, E. D.; Mandava, N. B., Eds.; CRC Press: Boca Raton, USA, 1988; Vol. IV, Part B, pp. 80–110; (d) Fischer, N. H.; Olivier, E. J.; Fischer, H. D. *Fortschr. Chem. Org. Naturst.* **1979**, *38*, 47–390; (e) Devon, T. K.; Scott, A. I. *Handbook of Naturally Occurring Compounds*; Academic Press: New York, 1975; Vol. 1.
2. (a) Trost, B. M.; Tang, W.; Schulte, J. *Org. Lett.* **2000**, *2*, 4013–4015; (b) Singh, I. P.; Milligan, K. E.; Gerwick, W. H. *J. Nat. Prod.* **1999**, *62*, 1333–1335; (c) Gunasekera, S. P.; Gunasekera, M.; Longley, R. E.; Shulte, G. K. *J. Org. Chem.* **1990**, *55*, 4912–4915; (d) Rocca, J. R.; Tumlinson, J. H.; Glancey, B. M.; Lofren, C. S. *Tetrahedron Lett.* **1983**, *24*, 1893–1896 and 1889–1892; (e) Endo, A.; Kuroda, M.; Tsujita, Y. *J. Antibiot.* **1976**, *29*, 1346–1348.
3. (a) Lin, G.-Q.; Wang, W.; Xu, M.-H. *Org. Lett.* **2000**, *2*, 2229–2232; (b) Miyabe, H.; Fujii, K.; Goto, T.; Naito, T. *Org. Lett.* **2000**, *2*, 4071–4074; (c) El Ali, B.; Alper, H. *Synlett* **2000**, 161–171; (d) Cardillo, G.; Tommasini, C. *Chem. Soc. Rev.* **1996**, *25*, 117–128; (e) Matsushima, A.; Kodera, Y.; Hiroto, M.; Nishimura, H.; Inada, Y. *J. Mol. Cat. B: Enzymatic* **1996**, *2*, 1–17; (f) Cole, D. C. *Tetrahedron* **1994**, *50*, 9517–9582.
4. (a) Faber, K. *Biotransformations in Organic Compounds*; Springer: Berlin, 2000; (b) Santaniello, E.; Ferraboschi, P.; Grisenti, P.; Manzocchi, A. *Chem. Rev.* **1992**, *92*, 1071–1140; (c) Csuk, R.; Glänzer, B. I. *Chem. Rev.* **1991**, *91*, 49–97; (d) Servi, S. *Synthesis* **1990**, 1–25.
5. (a) Aragozzini, F.; Maconi, E.; Craveri, R. *Appl. Microbiol. Biotechnol.* **1986**, *24*, 175–177; (b) Fantin, G.; Fogagnolo, M.; Giovannini, P. P.; Medici, A.; Pedrini, P.; Gardini, F.; Lanciotti, R. *Tetrahedron* **1996**, *52*, 3547–3552.
6. Molinari, F.; Gandolfi, R.; Villa, R.; Occhiato, E. G. *Tetrahedron: Asymmetry* **1999**, *10*, 3515–3520.
7. Gessner, M.; Günther, C.; Mosandl, A. *Z. Naturfortsch.* **1987**, *42c*, 1159–1164.
8. Utaka, M.; Watabu, H.; Takeda, A. *J. Org. Chem.* **1987**, *53*, 4363–4368.
9. Dufosse, L.; Feron, G.; Latrasse, A.; Guichard, E.; Spinnler, H.-E. *Chirality* **1997**, *9*, 667–671.
10. Benedetti, F.; Forzato, C.; Nitti, P.; Pitacco, G.; Valentin, E.; Vicario, M. *Tetrahedron: Asymmetry* **2001**, *12*, 505–511.
11. Heide, R.; de Valois, P. J.; Visser, J.; Jaegers, P. P.; Timmer, R. In *Analysis of Food and Beverages*; Charalambous, G., Ed.; Academic Press: New York, 1978; p. 275.
12. Manzocchi, A.; Casati, R.; Fiecchi, A.; Santaniello, E. *J. Chem. Soc., Perkin Trans. 1* **1987**, 2753–2757.
13. Hanessian, S.; Raghavan, S. *Biorg. Med. Chem. Lett.* **1994**, *4*, 1697–1702.
14. Ganaha, M.; Funabiki, Y.; Motoki, M.; Yamauchi, S.; Kinoshita, Y. *Biosci. Biotechnol. Biochem.* **1998**, *62*, 181–184.
15. Fogal, E.; Forzato, C.; Nitti, P.; Pitacco, G.; Valentin, E. *Tetrahedron: Asymmetry* **2000**, *11*, 2599–2614.

16. Felluga, F.; Nitti, P.; Pitacco, G.; Valentin, E. *Gazz. Chim. Ital.* **1993**, *123*, 443–447.
17. Forzato, C.; Nitti, P.; Pitacco, G.; Valentin, E. *Tetrahedron: Asymmetry* **1999**, *10*, 1243–1254.
18. (a) Egorova, A. Y.; Sedavkina, V. A.; Morozova, N. A. *Chem. Heterocyclic Compd.* **1999**, *35*, 41–44; (b) Hooz, J.; Layton, R. B. *Can. J. Chem.* **1972**, *50*, 1105–1107.
19. Drioli, S.; Felluga, F.; Forzato, C.; Nitti, P.; Pitacco, G. *Chem. Commun.* **1996**, 1289–1290.
20. Patrick, Jr., T. M. *J. Org. Chem.* **1952**, *17*, 1009–1016.
21. (a) Kohno, Y.; Narasaka, K. *Bull. Chem. Soc. Jpn.* **1995**, *68*, 322–329; (b) Yasuda, M.; Oh-hata, T.; Shibata, I.; Baba, A.; Matsuda, H. *J. Chem. Soc., Perkin Trans. 1* **1993**, 859–865; (c) Rieke, R. D.; Stack, D. E.; Dawson, B. T.; Wu, T.-C. *J. Org. Chem.* **1993**, *58*, 2483–2491; (d) Rieke, R. D.; Wehmeyer, R. M.; Wu, T.-C.; Ebert, G. W. *Tetrahedron* **1989**, *45*, 443–454; (e) Degl'Innocenti, A.; Ricci, A.; Mordini, A.; Reginato, G.; Colotta, V. *Gazz. Chim. Ital.* **1987**, *117*, 645–648.
22. Blanco, L.; Rousseau, G.; Barnier, J.-P.; Guibé-Jampel, E. *Tetrahedron: Asymmetry* **1993**, *4*, 783–792.
23. (a) House, H. O.; Tefertiller, B. A.; Olmstead, H. D. *J. Org. Chem.* **1968**, *33*, 935–942; (b) Karady, S.; Lenfant, M.; Wolff, R. E. *Bull. Soc. Chim. Fr.* **1965**, 2472–2474; (c) Stork, G.; Brizzolara, A.; Landesman, H.; Szmuszkovicz, J.; Terrel, R. *J. Am. Chem. Soc.* **1963**, *85*, 207–222.
24. Cotarca, L.; Delogu, P.; Maggioni, P.; Nardelli, A.; Bianchini, R.; Sguassero, S. *Synthesis* **1997**, *3*, 328–332.
25. Schick, H.; Ludwig, R. *Synthesis* **1992**, 369–370.
26. Frenette, R.; Kakushima, M.; Zamboni, R.; Young, R. N.; Verhoeven, T. R. *J. Org. Chem.* **1987**, *52*, 304–307.
27. Sucrow, W.; Slopianka, M.; Winkler, D. *Chem. Ber.* **1972**, *105*, 1621.
28. Green, S. P.; Witting, D. A. *J. Chem. Soc., Perkin Trans. 1* **1996**, 1027–1034.
29. Podraza, K. F. *J. Heterocyclic Chem.* **1987**, *24*, 293–295.
30. (a) Reissig, H.-U.; Angert, H. *J. Org. Chem.* **1993**, *58*, 6280–6285; (b) Ueno, Y.; Moriya, O.; Chino, K.; Watanabe, M.; Okawara, M. *J. Chem. Soc., Perkin Trans. 1* **1986**, 1351–1356; (c) Fang, J.-M.; Liao, L.-F.; Hong, B.-C. *J. Org. Chem.* **1986**, *51*, 2828–2829; (d) Nakamura, E.; Oshino, H.; Kuwajima, I. *J. Am. Chem. Soc.* **1986**, *108*, 3745–3755; (e) Fristad, W. E.; Peterson, J. R. *J. Org. Chem.* **1985**, *50*, 10–18.
31. Griffiths, D. V.; Wilcox, G. *J. Chem. Soc., Perkin Trans. 2* **1988**, 431–436.